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Efavirenz Mannich bases: Synthesis, anti-HIV and antitubercular activities

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Abstract

A series of efavirenz Mannich bases has been synthesized by reacting efavirenz, formaldehyde, and various aryl substituted piperazines using microwave irradiation (yield 35–88%). The synthesized compounds were evaluated for *in-vitro* anti-HIV and antimycobacterial activities. The *in-vitro* antiretroviral activities indicated that compound 7-(4-((6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2-oxo-2H-benzo[d] [1,3]oxazin-1 (4H)-yl)methyl)-3-methylpiperazin-l -yl)-1-cyclopropyl-6-fluoro-l,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (**4i**) was equipotent to efavirenz with EC_{50} of 2.4 nM. Compound **4i** also inhibited *M. tuberculosis* with minimum inhibitory concentration of 0.2 μg/mL.

Keywords: *efavirenz, Mannich bases, anti-HIV activity, antitubercular activity*

Indroduction

Acquired immunodeficiency syndrome (AIDS) is caused by the retrovirus, human immuno deficiency virus (HIV) [1]. The number of people living with HIV continues to grow and around 40 million peoples are infected with HIV globally, and over 20 million have died since the first cases of AIDS were identified in 1981 [2]. The HIV infection, which targets the monocytes expressing surface CD4 receptors, eventually produces profound defects in cell-mediated immunity [3]. Overtime infection leads to severe depletion of CD4 T-lymphocytes (T-cells) resulting in opportunistic infections (OI) like tuberculosis (TB), fungal, viral, protozoal and neoplastic diseases and ultimately death. Worldwide, TB is the most frequent co-infection in subjects with HIV type 1 infection [4]. HIV-1 infection remains the most common risk factor for the development of active TB [5]. Both reactivation of a latent *Mycobacterium tuberculosis* (MTB) infection and progressive primary TB are substantially more common in HIV-1-infected subjects [6]. The development of active TB accelerates the progression of HIV disease towards full-blown AIDS, accompanied by enhanced HIV replication. Through rational thinking, it appears that an ideal drug for HIV/AIDS patients should suppress HIV replication thereby acting as anti-HIV drug and also should treat OI like TB [7–8]. In continuation of our work on anti-HIV prodrugs [9–13], we undertook a study to prepare and evaluate efavirenz prodrugs in an effort to identify compounds which could suppress HIV-replication and also inhibit MTB.

Materials and Methods

Chemistry

A domestic microwave oven with the following specifications has been used: make LG; input 220 V ~ 50 Hz, 980 W, 4.7°A; frequency 2450 MHz. Melting points were determined in open capillary tubes on a Büchi 530 melting point apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded for the

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compounds on a Jasco IR Report 100 (KBr) and Brucker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. Elemental analyses (C, H, and N) were undertaken with a Perkin-Elmer model 240C analyzer. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silicagel-G (Merck) coated aluminium plates, visualized by iodine vapour. Developing solvents were chloroform-methanol (9:1).

Synthesis of Compounds 4a-i. The general procedure for preparing **4a-i** was as follows. To a solution of (6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-lH-benzo[d][l,3]oxazin-2-(4H)-one) (efavirenz, 0.02 mol) in absolute ethanol (50 mL), was added various aryl piperazine derivatives (0.02 mol) and 37% formalin (1 mL). The reaction mixture was irradiated in an unmodified domestic microwave oven at 80% intensity with 30 s/cycle for 3 min and set aside. The resultant solid was washed with dilute ethanol dried and recrystallized from ethanol-chloroform mixture. Yield 35–88%.

Spectral and elemental analysis data for representative compounds

*6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-l-((4-phenylpiperazin-l-yl)methyl)-lH-benzo[d][l,3]oxazin-2(4H)-one (**4a**):* IR (KBr): 3010, 2862, 2840, 1742, 1640, 1620, 1506, 1240, 1125 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.7–0.82 (m, 4H, cyclopropyl-H of efavirenz), 1.58 (m, IH, cyclopropyl-H of efavirenz), 2.6–3.45 (m, 8H, -piperazine-H), 4.0 (s, 2H, -NCH_2N), 5.1 (m, IH, CH of ethylene linker attached to cyclopropyl), 6.0 (d, IH, CH of ethylene linker attached to benzoxazine), 6.59–7.54 (m, 8H, Ar-H); Calculated for $C_25H_{25}C1F_3N_30_2$: C, 61.04; H, 5.12; N, 8.54. Found: C, 61.01; H,4.15;N, 8.59%.

*6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-l-((4-(4-methoxyphenyl)piperazin-1 -yl)methyl)-l H-benzo[d][1,3]oxazin-2(4H)-one (**4c**):* IR (KBr): 3010, 2860, 2842, 1742, 1640, 1620, 1510, 1240, 1125 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.7–0.80 (m, 4H, cyclopropyl-H of efavirenz), 1.58 (m, IH, cyclopropyl-H of efavirenz), 2.62–3.46 (m, 8H, -piperazine-H), 3.73 (s, 3H, methoxy), 4.02 (s, 2H, -NCH_2N), 5.1 (m, IH, CH of ethylene linker attached to cyclopropyl), 6.02 (d, IH, CH of ethylene linker attached to benzoxazine), 6.6–7.54 (m, 7H, Ar-H); Calculated for $C_{26}H_{27}CIF_3N_3O_3$: C, 59.83; H, 5.21; N, 8.05. Found: C, 59.90; H, 5.19; N, 8.09%.

*7-(4-((6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2-oxo-2H-benzo[d] [1,3]oxazin-1 (4H)-yl) methyl)-3 -methylpiperazin-1 -yl)-6,8-difluoro-1 -ethyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**4f**):* IR (KBr): 3010, 2860, 2846, 1740, 1640, 1620, 1506, 1236, 1125 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.7–0.82 (m, 4H, cyclopropyl-H of efavirenz), 1.28 (t, 3H, CH_3 of C_2H_5), 1.58 (m, IH, cyclopropyl-H of efavirenz), 2.7–3.6 (m, 8H, -piperazine-H), 4.03 (s, 2H, -NCH_2N), 4.25 (q, 2H, CH_2 of C_2H_5), 5.1 (m, IH, CH of ethylene linker attached to cyclopropyl), 6.0 (d, IH, CH of ethylene linker attached to benzoxazine), 6.60–7.51 (m, 5H, Ar-H), 8.0 (s, IH, C_2-H), 14.60 (bs, IH, COOH); Calculated for $C_{31}H_{29}CIF_4N_4O_5$: C, 57.37; H, 4.50; N, 8.63. Found: C, 57.39; H, 4.49; N, 8.60%.

*7-(4-((6-chloro-4-(2-cycIopropylethynyl)-4-(trifluoromethyl)-2-oxo-2H-benzo[d][l,3]oxazin-l(4H)-yl)methyl)-3-methylpiperazin-l-yl)-l-cyclopropyl-6-fluoro-l,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (**4i**):* IR (KBr): 3010, 2860, 2846, 1740, 1640, 1620, 1506, 1236, 1125 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.28–0.53 (m, 4H, cyclopropyl-H of quinolone), 0.7–0.82 (m, 4H, cyclopropyl-H of efavirenz), 1.2 (s, 3H, CH_3 of piperazine), 1.4 (m, IH, cyclopropyl-H of quinolone), 1.58 m, IH, cyclopropyl-H of efavirenz), 2.6–3.4 (m, 7H,-piperazine-H), 3.73 (s, 3H, methoxy), 4.03 (s, 2H, -NCH_2N), 5.1 (m, IH, CH of ethylene linker attached to cyclopropyl), 6.0 (d, IH, CH of ethylene linker attached to benzoxazine), 6.60–7.7 (m, 4H, Ar-H), 8.0 (s, IH, C_2-H), 14.86 (bs, IH, COOH); Calculated for $C_{34}H_{33}CIF_4N_4O_6$:C, 57.92; H, 4.72; N, 7.95. Found: C, 57.91; H, 4.69; N, 7.90%.

Anti-HIV activity

The compounds were tested for anti-HIV activity against replication of HIV-1 (III B) in CEM cells [14]. The CEM cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow lab, Irvine Scotland), supplemented with 10% (v/v) heat-inactivated calf serum and 20-μg/mL gentamicin (E. Merck, Darmstadt, Germany). HIV-1 (III B) was obtained from the culture supernatant of HIV-1 infected MT-4 cell lines and the virus stocks were stored at −70° C until used. Anti- HIV assays were carried out in microtitre plates filled with 100 μL of medium and 25 μL volumes of compounds in triplicate so asto allow simultaneous evaluation of their effects on HIV and mock infected cells. Fifty microlitres of HIV at 100 $CCID_{50}$ (50% cell culture infective dose) medium were added to either the HIV infected or mock infected part of the microtitre tray. The cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. Five days after infection the viability of mock and HIV-infected cells were examined spectrophotometrically by using the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [8].

In vitro *antimycobacterial activity (Agar dilution method) [15]*

Compounds were evaluated for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv

procured from the Tuberculosis Research Center, Indian Council of Medical Research, Chennai, India. The agar dilution method was performed using Middlebrook 7H10 medium supplemented with Middlebrook OADC medium (Hi-Media). After solidification of the agar, the plates were inoculated with 0.1 mL of 10^{-2} and 10^{-4} dilutions of a McFarland 1.0 concentration of a suspension of organism. The inoculated plates were then incubated at 37° C for 4 weeks. The minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculums.

In vitro *stability studies [16]*

To 990 μL of phosphate buffer (0.2 M, pH 7.4) was added 10 μL of a solution of appropriate drugs (10 mg/mL in dimethyl sulfoxide) and the mixture was incubated at 37 °C in a water bath. At various time intervals (0–4 h), 100 μL of the samples were withdrawn and added immediately to ice-cold methanol (400 μL). The samples were centrifuged and the supernatants were filtered through nylon 66 filters (0.45 pm) and analyzed by spectrophotometrically. From the observed absorbance changes, at various wavelengths, the half-lives of the analogues were calculated.

Results and Discussion

Synthesis and Characterisation

Efavirenz (6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-lH-benzo[d] [1,3] oxazin-2-(4H)-one), is a very potent HIV-1 non-nucleoside reverse transcriptase inhibitor [17]. T Toll date, efavirenz has been modified at the 4-, 5- and 6-positions of the benzoxazine moiety [18], and in this work we modified the amide hydrogen at the 1-position. The conversion of compounds containing an active hydrogen atom to the corresponding Mannich bases has been used extensively in the molecular modification approach [19]. Here we prepared the Mannich bases of efavirenz by reacting it with formaldehyde and secondary amine function of substituted piperazines utilizing microwave irradiation to form the required efavirenz derivatives in 35–88% yield. The general proceduce for the preparation of target compounds **4a–4i** (Table I) is described in Scheme 1. Unlike conventional methods (duration-5 h) [17], microwave-assisted reactions were very facile (3-4 min), and the products do not require any further purification. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) and elemental analyses and the structures were confuted by spectral data. In general, infra red spectra (IR) revealed a CH_2 (Mannich methylene) peak at 2860 and 2846 cm^{-1}. In the ^1H NMR spectra the signals of the respective protons of the prepared efavirenz derivatives were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet at δ 4.0–4.03 ppm corresponding to the-NCH_2N- group; a multiplet at 0.7–0.82 and 1.58 ppm for the cyclopropyl proton; a multiplet at δ 2.59–3.45 ppm for the piperazine proton; a multiplet at δ 6.99–7.4 ppm for the aromatic protons. The absence of a D_2O exchangeable singlet at 11.05 ppm indicated that the active hydrogen of efavirenz was substituted with the aminomethyl group. The elemental analysis results were within ± 0.4% of the theoretical values.

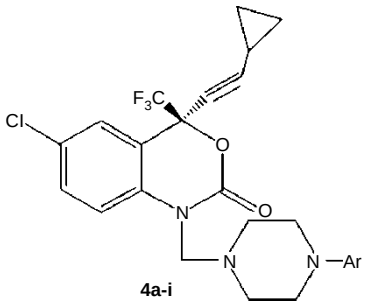
Biological investigation and discussion

The synthesized compounds were evaluated for their inhibitory effect on the replication of HIV-1 in CEM cell lines and their EC_{50} (effective concentration of compound (nM) achieving 50% protection in CEM lines against the cytopathic effect of HIV-1), and CC_{50} (cytotoxic concentration of compound (μM) required to reduce the viability of mock infected CEM cells by 50%), are reported in the Table l along with efavirenz as standard drug for comparison. Examination of the obtained results revealed that compounds **4a–4i** exhibited excellent anti-HIV activity. Among the synthesized compounds, three compounds (**4e**, **4f**, **and 4i**) were found to be equally active as efavirenz. Compound **4i** was found to be the most potent compound with EC_{50} of 2.4 nM and CC_{50} of >10 μM with selectivity index (CC_{50}/EC_{50}) of >4166.

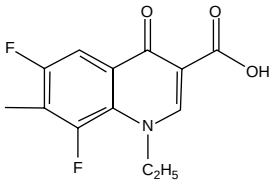
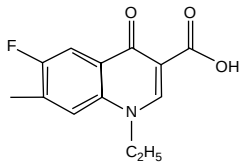
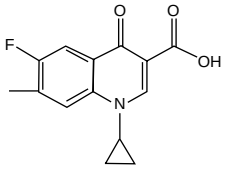
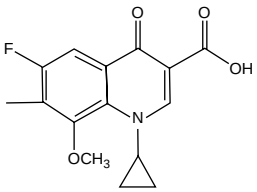
All the compounds were screened for their antimycobacterial activity against MTB by the agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards [15] for the determination of minimum inhibitory concentration (MIC). The MIC was defined as the minimum concentration of compound required to inhibit complete growth of bacteria and MIC's of the compounds were reported in Table I. Among the derivatives tested, compounds bearing the fluroquinolone moiety were found to be promising and compound **4i** was found to the most active compound with MIC of 0.2 μg/mL. This enhanced activity might be due to the inhibition of MTB DNA gyrase by the compounds **4f-i** [20].

The usefulness of the prodrugs of efavirenz should depend not only on the stability of the prodrug for its transport across the cell membrane but also upon its reversion to the parent compound intracellularly, especially in the virally infected cells. The half-lives ($t_{1/2}$) of hydrolysis of the prodrugs **(4e-f, 4i)** were therefore determined at pH 7.4, 37°C. The data in Table I indicated that the various prodrugs of efavirenz were susceptible to hydrolysis with $t_{1/2}$ in the range of 120–240 min through deaminomethylation to release efavirenz.

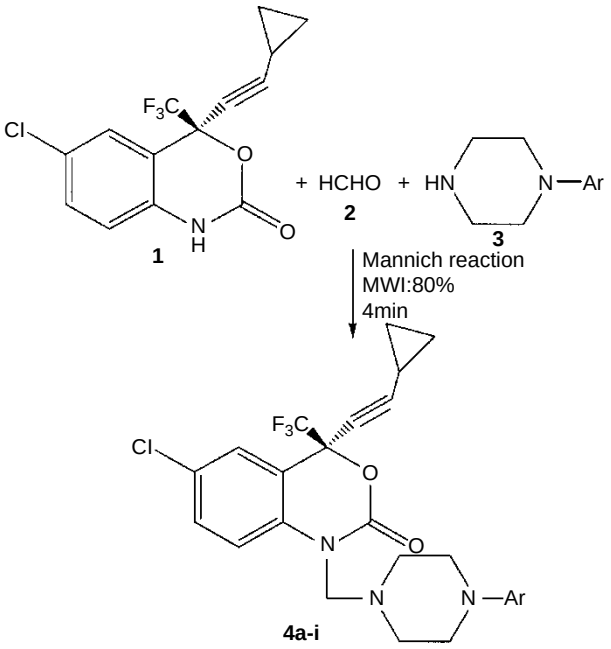
Table I. Physical constants and biological activities of efavirenz Mannich bases.



4a-i

Comp.	Ar	Yield%	m.p.°C	logP[a]	EC_{50}^{b} (nM)	CC_{50}^{c} (μM)	MIC MTB (μg/ML/)[d]	$t_{1/2}$ (min)[e]
4a	Phenyl	45	90	6.27	3.0	>10	>12.5	ND
4b	2-Pyridyl	36	105	5.65	12.1	NT	>12.5	ND
4c	4-Methoxyphenyl	35	119	6.15	6.69	NT	>12.5	ND
4d	4-Fluoro phenyl	46	122	6.43	3.6	NT	>12.5	ND
4e	3-Chloro phenyl	52	113	6.83	2.4	>10	>12.5	120
4f		85	155	6.42	2.58	>10	3.12	240
4 g		88	152	5.94	3.79	NT	1.56	ND
4 h		70	187	5.9	4.47	NT	0.4	ND
4i		78	197	6.09	2.4	>10	0.2	180
Efa	–	–	–	3.93	2.53	>10	>12.5	

[a]logP values calculated with Chem Office 2004 software, [b]effective concentration of compound achieving 50% protection in MT-4 cell lines against the cytopathic effect of HIV-1, [c]CC_{50} cytotoxic concentration of compound required to reduce the viability of mock infected CEM cells by 50%, [d]minimum inhibitory concentration against *M. tuberculosis*, [e] At pH 7.4 at 37°C, **4f**, **4i** contains group at piperazine moiety. NT-Not tested. ND- Not determined.



Scheme 1. Protocol for the synthesis of efavirenz prodrugs.

This study revealed that compound **4i** was found to be a promising compound for the treatment of AIDS, as shown by excellent anti-HIV and antimycobacterial activity. Compound **4i** suppresses HIV replication thereby acting as an anti-HIV drug and also could be useful to treat OI like TB.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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